（学位第３号様式）

学　位　論　文　要　旨	
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題　目	Analysis of factors that influence the fermentation profiles of sake yeast **(日本酒酵母の発酵特性に及ぼす影響の解析)**

In past few decades various factors has been used for developing fermentation profile of yeast. Among them, use of additional substances during fermentation affect the several physiological properties of yeast. Hence, this study was implemented to reveal the effects of the additional substances such as glycosylceramide (GlcCer) and amino acids on the fermentation profiles of sake yeast.

Until date, sake yeast is fermented with koji, steamed rice fermented with non-pathogenic fungus *Aspergillus oryzae* for the manufacture of sake. However, *A. oryzae* contain abundant GlcCer as cell membrane component. In this study, we first report a novel and efficient method for quantitation of mycelial weight of *A. oryzae* by measuring its GlcCer content. Previously reported that *N*-acetylglucosamine is used to quantitate the mycelial weight of *A. oryzae* which is costly, tedious and its quantitative performance is low. Therefore, in this study the results suggested that the amount of GlcCer significantly correlated with both the mycelial weight of *A. oryzae* and the amount of *N*-acetylglucosamine, an established index of the mycelial weight of *A. oryzae* in koji. This new method is simple, rapid and reproducible.

In this study, we also evaluated that, GlcCer, one of the sphingolipids affects yeast fermentation. During fermentation, yeast requires unsaturated fatty acids and sterols, in addition to substances provided by koji enzymes. However, the role of sphingolipids on the brewing characteristics of sake yeast has not been studied. The addition of soy, *A. oryzae*, and *G. frondosa* GlcCer conferred similar effect on the flavor profiles of sake yeast. In particular, the addition of *A. oryzae* and *G. frondosa* GlcCer were very similar in terms of the decreases in ethyl caprylate and ethyl 9-decenoate. Addition of soy GlcCer induced metabolic changes to sake yeast such as decrease in glucose, increases in ethanol and glycerol and also changes in several amino acids and organic acids concentrations. TCA cycle, pyruvate metabolism, starch and sucrose metabolism, and glycerolipid metabolism were overrepresented in the cultures incubated with sake yeast and soy GlcCer which is first study in this research.

The brewing profiles of yeast are also affected by the addition of amino acids during alcoholic fermentation. For example, addition of methionine decreases hydrogen sulfide production by brewery yeasts. However, a concrete mechanism linking amino acids in fermentation media with mitochondrial activity during fermentation of brewery yeasts is yet unknown. Here, we reported that amino acids in fermentation media, especially methionine (Met) and glycine (Gly), stabilize mitochondrial activity during fermentation of sake yeast. By utilizing *atg32Δ* mutant sake yeast, which shows deteriorated mitochondrial activity, we screened candidate amino acids Met and Gly by measuring reactive oxygen species (ROS) levels in sake yeast. Yeast cells supplemented with Met and Gly retained high ROS levels relative to the non-supplemented sake yeast. Moreover, Met-supplemented cells showed a metabolome distinct from that of non-supplemented cells.

In conclusion, we have first determined the altered fermentation characteristics of sake yeast in response to glycosylceramide and amino acids which will enable interpretation on the fermentation characteristics of yeast.